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BIOLOGICAL BULLETIN

EXPERIMENTAL ALTERATION OF THE AXIAL GRADIENT IN THE ALGA, GRIFFITHSIA BORNETIANA.

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(WITH 15 FIGURES.)

The existence of axial gradients in susceptibility to various agents and conditions has been demonstrated as a characteristic feature of axiate animals, and the relation of such gradients on the one hand to metabolic and protoplasmic condition and on the other to the morphological and physiological order or pattern characteristic of development has been discussed in various publications.¹ The results of these investigations on animals led to the attempt to determine whether similar axial gradients occur in plants and some of the data on algæ have already appeared (Child, '16c, '16e, '17). The existence of such gradients in the axes of at least certain plants as well as in the axes of animals being demonstrated, it remains to determine their relation to the order or pattern of the plant axis. To accomplish this completely it would be necessary to obliterate or reverse existing gradients, to determine experimentally the origin of new gradients and to observe the effect of such changes upon order or pattern in the plant body.

Obliteration and reversal of axial gradients in algæ by various means have already been described (Child, '16e, '17). In most cases these changes represented a stage in the process of dying, and while they showed that changes might occur, the fact that these changes were soon followed by death made it impossible

¹ See Child, '15a, Chap. IX., '15b, '15c, '16a, '16b, '16c, '16d. Hyman '16, and references to further literature in these publications.

to determine their effect upon the developmental and morphological order in the plant body. In the present paper some experimental alterations of the gradient which do not necessarily end in death are described, and some evidence concerning the effect of these changes on further development and axiation is presented. This is merely the first step toward the experimental determination of the relation between the axial gradient and polarity in the plant and the very simple experiments described here serve chiefly to point the way to more exact determination and control.

For experimental purposes a form with large cells and sensitive enough to react readily to environmental changes but not too sensitive was desirable. I am indebted to Professor Osterhout for calling my attention to *Griffithsia*, which has proved to be of interest in various ways.

THE GRIFFITHSIA AXIS AND GRADIENT.

The cell outline of an average, well-developed vegetative axis and branches from the material employed is indicated in Fig. 1, the hairs (Child, '17) being omitted. The method of growth is evident from the figure. The apical cell grows to a certain variable size and divides into a very small apical cell which repeats the process of growth and division, and a larger sub-apical cell, which continues to grow, but does not divide unless it later gives rise to a new axis by the separation of a new apical cell from some point of its apical region, as in the formation of new branches (Fig. 1). At a greater or less distance from the apical end a secondary basal elongation of the cells begins and is evident in the progressive change of shape of the cells toward the basal end of the axis (Fig. 1).

As already noted (Child '16e), the general susceptibility gradient in such an axis and in each branch is basipetal when the plant is in good condition. In the apical third or half of the length the gradient is very uniform, but in the basal region irregularities occur consisting in a susceptibility in one or more cells above or below the expectation corresponding to the level of the cell or cells. In the two or three most apical cells the intracellular gradient is usually slight or sometimes not visible,

but is basipetal when present. Often the apical cell itself shows a distinct basipetal gradient. Several cells from the third or fourth on usually show a distinct basipetal intracellular gradient, but with the beginning of the secondary basal elongation of the cell a basal region of high susceptibility usually appears, and some of these elongating cells show a double gradient, a region near the middle being the least susceptible. In these elongated cells of the more basal levels of well-developed axes the intracellular gradients may differ from cell to cell and from time to time, even though the general axial gradient remains essentially unaltered.

Not infrequently rhizoids develop on these more basal elongated cells even at a distance of several cells from the basal end of the axis, and in such cases the rhizoid may appear at any level, even close to the apical end of the cell, while the latter is still, at least physically, a part of the axis. In cases where such well-developed rhizoids are found on such cells it is of course impossible to determine the relation between the intracellular gradient and the point of origin of the rhizoid at the time when the rhizoid arose, and the possibility always exists that the intra-cellular gradient may have undergone alteration since the origin of rhizoid.

Rhizoid development is primarily a feature of the basal end or the more basal regions of the axes of algae (Tobler, '06), and as such we might expect to find it associated with regions of low susceptibility, *i. e.*, of low metabolic rate. The alterations in the intracellular gradients of the older more basal cells of *Griffithsia* suggest the possibility that even where a rhizoid arises from the middle or the apical region of such a cell this region may be the region of lowest metabolic rate in that particular cell.

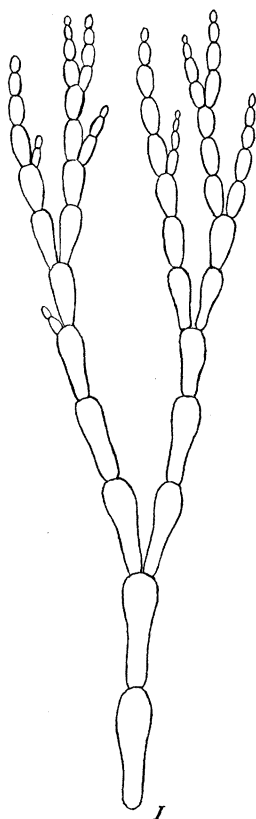


FIG. I.

The appearance of rhizoids or rhizoid-like structures on the older, more basal parts of the axes, is a characteristic feature of various species of algæ and in some cases these structures are so numerous as to form a more or less continuous cortical layer on such parts. In *Dasya*, for example, where such a secondary cortex formed by rhizoids is present on the older portions of the axes, the cells of this region show a relatively low susceptibility. In short, the rhizoid appears to be a structure originating at the lower levels of an axial metabolic gradient, though of course external conditions may determine whether or not rhizoids shall develop at levels where their development is possible.

Some of the experimental evidence presented below, as well as the alteration and reversal of the primary intracellular gradient in the more basal cells indicates very clearly that the degree of axiate individuation, *i. e.*, the permanence of the primary metabolic axial relations and their effectiveness in maintaining a particular order is not very high. Moreover, the short length which the axes attain, the slight connection between the cells, the irregularity of branching and the above-mentioned development of rhizoids from any level of the more basal cells all support this conclusion.

If physiological polarity is primarily a metabolic gradient in protoplasm, such a form as *Griffithsia* should be favorable material for the experimental obliteration of old axes or gradients and the establishment of new ones. The experiments described below, which are suggestive rather than conclusive, constitute a first step in this direction.

EXPERIMENTAL OBLITERATION AND REVERSAL OF THE GRADIENT IN RELATION TO INDIVIDUATION.

The fact that obliteration or reversal of the general axial gradient of *Griffithsia* usually occurs during the first few days in the laboratory, even where the plants are kept in running water, has been noted in an earlier paper (Child, '16*e*). The results of a few simple experiments along this line remain to be considered.

Confinement in a limited supply of water without change in a closed flask without air was used as an extreme degree of "bad"

environmental conditions. In these experiments masses consisting of many axes were placed in 100 c.c. Erlenmeyer flasks which were filled with water, corked without air space and kept in the running water of the laboratory aquarium under the same conditions of light and temperature as the stock.

Susceptibility tests were made on the fresh material before confinement, and at intervals during the experiment the susceptibility to KCN $m/100$ or $m/50$ of portions removed from the flask was compared with that of controls from the same plants kept in running water, the phycoerythrin serving as indicator of death, as described in an earlier paper (Child, '16e).

The results of these experiments are briefly as follows: the plants of course die in the flask in a few days at most, in my experiments in 2-7 days, the survival time varying in general inversely as the amount of plant tissue present. The death gradient is basipetal as with other agents, *i. e.*, the apical regions are most susceptible to the confinement and the susceptibility decreases basipetally. The progress of death can be observed with the naked eye without difficulty, for each cell as it dies becomes orange-yellow by reflected light.

After twenty-four hours' confinement and before the death of any cells the susceptibility to KCN has decreased and the general susceptibility gradient in KCN shows a more or less complete reversal in the apical regions of most axes. This reversal may involve only the apical cell itself, in which case it dies later instead of earlier than the cell next below it, or it may include two, three or even the five or six most apical cells of the axis. In such cases these cells are less susceptible and die later than the cells next below, and in this group the death gradient from cell to cell is more or less distinctly acropetal, the apical cell dying last. Where the apical cell has recently divided and is very small it may still show a high susceptibility and the reversal may occur in the subapical region.

After forty-eight to sixty hours' confinement the susceptibility to KCN is usually again as high as or even considerably higher than that of fresh plants and the gradient is in general again basipetal. This increase in susceptibility and the reappearance of the basipetal gradient in KCN is soon followed in my experiments by the beginning of death in the apical regions.

This apparent double reversal in the susceptibility gradient and the decrease followed by increase in susceptibility to KCN requires some consideration. This case illustrates the difficulties which immediately arise when susceptibility to one inhibiting agent or condition is used as the measure of the action of another. Until we know more concerning the changes preceding death in such a case as this, it is impossible to reach any definite conclusion concerning the meaning of the changes in susceptibility, but certain probabilities suggest themselves.

In the first place the decrease in susceptibility and the more or less complete reversal of the original gradient, the same changes which have been observed in various species and under various depressing or injurious conditions (Child, '16e, '17), undoubtedly indicate a differential depression or injury along the axis, the most susceptible, apical regions being so much more injured than less susceptible, more basal regions, that the original relations are often reversed. The point of interest is that changes of this sort brought about by high temperature, confinement, etc., appear in the altered susceptibility gradient to KCN. This apparently means that the action of these other conditions and that of KCN on the protoplasm are not exactly of the same sort, *i. e.*, not strictly additive, for if they were, we should expect the KCN simply to continue the action of the high temperature or confinement without making them visible as a reversed gradient and a decreased susceptibility.

As death approaches, however, at least in the confinement experiments, further changes apparently occur which differ from those of the earlier stages and these changes appear in increased susceptibility to KCN and a second reversal of the susceptibility gradient, a return to the normal basipetal condition. Under ordinary conditions such increase in susceptibility would be interpreted as associated with an increase in metabolic rate, greatest in the apical regions, but it is impossible to believe that in the later stages of confinement the cells approaching death undergo any such increase in metabolic activity as these changes would indicate. It must be rather that in the later stages of the decrease in metabolism which precedes death under these conditions changes occur which are essentially like those pro-

duced by KCN so that plants at this stage behave as if they had already been subjected to KCN, and the final killing by KCN is simply or largely additive to the changes which have already occurred. The action of the ordinary narcotics such as alcohol and ether, at least in its more advanced stages, and that of KCN are also additive and thoroughly narcotized organisms are more susceptible to KCN than normal organisms, though there can be no question as to metabolic condition in the two cases. Evidently changes in susceptibility produced by one agent or condition can be made evident only by some agent or condition whose effect is not strictly additive to the first.

It seems not improbable that the present case is merely a particular example of a general relation. In terms of the permeability theory, for example, the first effect of an inhibiting agent may be, or be associated with a decrease in permeability, and if this effect is proportional to the original susceptibility along the axis, it is not difficult to understand that one such agent might produce a differential decrease in permeability and so reverse the axial gradient in susceptibility to another. In the later stages of the action of an inhibiting agent, there may be on the other hand an increase in permeability resulting from changes in protoplasmic aggregation or other changes, and after this stage is reached the effect of one agent is essentially additive to that of another. This is not to be regarded as a hypothesis in the scientific sense to account for the facts, but rather as merely a suggestion to illustrate how susceptibility relations may be altered. This case also shows certain of the limitations and difficulties of the susceptibility method in demonstrating by means of one agent the effects of another. Some of these difficulties may be removed or lessened by working with the proper combinations of agents.

For present purposes, however, the changes during the earlier, not those of the later stages of confinement are important. These changes, consisting in decrease in susceptibility and more or less reversal of the general gradient, are the same as the changes which occur very generally, though more slowly, under laboratory conditions even in running water. There can be no doubt that the transference of the plants from natural to labora-

tory conditions (Child, '16*e*), like the earlier stages of confinement, brings about a greater or less degree of physiological depression which is greatest in the most sensitive parts of the plant, the apical regions, and which, if sufficient, obliterates or reverses the original axial gradient. In cases of confinement in the small flasks as described, death usually follows this change in two or three days at most, but where the change occurs less rapidly, as in a large flask, in open standing water or in aquaria with running water, the obliteration or reversal of the gradient in the apical region is followed after two or three days by separation of the cells of the apical region in which the obliteration or reversal of the gradient occurred. In short, after obliteration or reversal of the gradient together with decrease in metabolic activity, the axis falls apart into its individual cells or into groups of two or three cells. This process begins apically because this is the region where the change first occurs, but as time goes on, if the environment does not improve, or if the plant is unable to become acclimated as a whole, the change in the gradient extends farther and farther in the basipetal direction and the separation into cells follows it in the same direction. In plants kept in open crystallizing dishes in a liter of sea-water without change, cell separation usually begins in the apical regions after several days and progresses basipetally, until after ten days most axes are completely separated into cells or small cell groups. In running water also cell separation usually begins in the apical regions after several days and progresses basipetally, though more slowly than in standing water or in a closed flask. In this case, however, separation often ceases at some level of the axis and the cells basal to this level maintain their continuity. The apical beginning, the basipetal progress, and under the less extreme conditions the halting at one level or another of this process of cell-separation constitute still another demonstration of the existence of an axial gradient in which susceptibility to these conditions is greatest apically and decreases basipetally.

This process of cell-separation is a reaction of the living cell, and is not necessarily followed by death, although under conditions in which any considerable degree of separation occurs, a varying percentage of the cells, chiefly the cells of more apical

regions, does die. In shallow crystallizing dishes with a liter of water fifty per cent. more or less of the cells may die, in more extreme conditions a higher percentage, up to one hundred per cent. in a closed flask, and under less extreme conditions a smaller percentage.

In all cases, however, where conditions are not too extreme a certain percentage of the cells may remain alive, and after a time resume growth and development. In short, these depressing conditions if not too extreme may be used as a method of obtaining isolated cells of *Griffithsia* for purposes of culture. This method is not new, for Tobler, '02, '04, has employed it for this purpose, but the fact that such cell-separation is associated with obliteration or reversal of the axial gradient is new. If, as I believe (Child, '15*b*), the axial gradient is the basis of physiological polarity, and at the same time the primary integrating factor in axiate individuation, the obliteration or reversal of the gradient must mean both obliteration or reversal of the original polarity and physiological disintegration of the individual, in other words, the elimination of the physiological order or pattern associated with and resulting from the existence of the gradient. With this physiological disintegration of the individual its component parts, the cells, become more or less completely physiologically isolated (Child, '15*b*). The fact that physiological disintegration of the individual is soon followed by physical separation of the cells is of great interest, as indicating the very direct dependence in this simple individual, of the gross morphological order upon the dynamic integrating factor.

Moreover, if the gradient is the basis of physiological polarity, we may expect that the further growth and development of these isolated cells will be influenced in one way or another by the obliteration or reversal of the original gradient. If the original gradient is completely or to a large extent obliterated in a cell, a new gradient or gradients may be established by "chance" differentials in external conditions, or may be determined experimentally. If reversal of the original gradient persists for any considerable time, the order and axiation in the further development may be determined by this factor. In the following section the development of some of these isolated cells is described, and its relation to susceptibility gradients demonstrated.

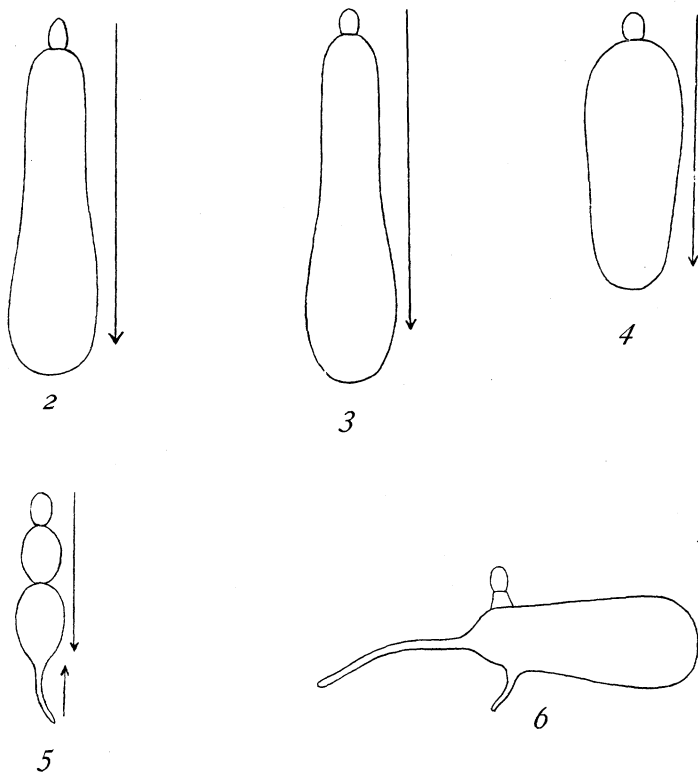
THE DEVELOPMENT OF NEW AXES FROM ISOLATED CELLS IN
RELATION TO THE GRADIENT.

One lot of *Griffithsia*, kept in running water for three days after collection, was then placed in a shallow crystallizing dish in one liter of water and left open to the air in diffuse daylight without change of water. After two days in standing water cell separation began apically, and after seven days had extended over the whole length of the axes. Most of the cells were single, but a few groups of two, three or even four cells were observed. About half of the cells were dead at this time, the death rate being highest among the cells from more apical regions. Almost every one of the apical cells and probably 75-80 per cent. of the first three or four subapical cells were dead. Taking the shape and size of the isolated cells as an index of their approximate level in the axis, it is evident that the proportion of cells killed is greatest in the most apical regions and decreases basally, at least, to the middle regions of the axis, below which the gradient is slight, even under normal conditions. In short, the susceptibility gradient of the axis is in evidence under these conditions as well as in more rapid killing.

After another week in standing water, examination showed new growth and development in 10-15 per cent. of the living cells. Figs. 2 and 3 show cells from the more basal levels with new apical cells at what were originally the basal ends. Susceptibility gradients were determined in both these cases, the progress of death being in the direction of the arrows, the new apical cell dying before the old, large cell and the intracellular gradient in the large cell being in the direction of the arrow in both cases. In many other similar cases similar gradients were found and still other cases were observed without determination of the susceptibility gradient.

Fig. 4 shows a cell from the middle region of an axis, in which a new apical cell has arisen at the original apical end. The susceptibility gradient, both general and intracellular, is indicated by the arrow which points in the direction in which death progresses. Fig. 5 is a case in which a group of three cells from a level near the apical end have remained connected. The only new growth in this case is a rhizoid from the basal end of the

most basal cell of the group. Here the original susceptibility gradient persists in the three cells, as indicated by the arrow, while the rhizoid, like any other rhizoid, represents a new gradient in the opposite direction. The rhizoid, however, represents a



lower level of susceptibility than other parts of the individual and is the last portion to die.

In Figs. 2 and 3 the original gradient has undergone reversal, in Figs. 4 and 5 it still persists. Cases of reversal, like Figs. 2 and 3, were in general more frequent in the material than cases of persistence. This is to be expected, since cell separation is associated with obliteration or reversal of the gradient. The original gradient may persist in cells or cell-groups in which the susceptibility is for any reason exceptionally low and the differential injury consequently less marked, and probably also in cases of partial acclimation (Child, '15*a*, Chap. III., '16*d*) after

complete obliteration. It may be also that in some cells, where the original gradient had undergone reversal in connection with the basal growth, another reversal may accompany cell separation.

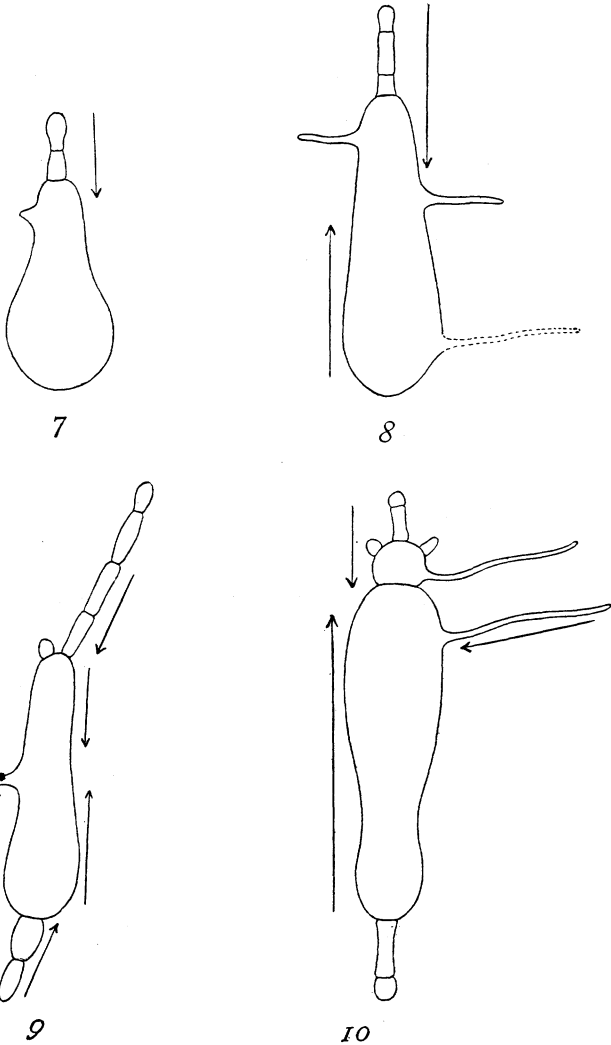
Fig. 6 shows a case in which a new polarity at right angles to the old appears in the basal region of a large cell. A new axis consisting of an apical and a subapical cell, has arisen from the lateral cell-surface near the basal end. The susceptibility gradient was not determined in this case, but there can be no doubt that the new axis represents a new gradient. The gradient in this case may perhaps have been directly determined by the differential action of an external factor, possibly concentration of oxygen or other substances necessary for metabolism. Cells lying on the bottom of the dish, or perhaps in contact with other cells, may often be subjected to such an external differential, and where the original gradient is to a large extent obliterated, a new gradient, and so a new axis may readily arise. Cases of this sort have been recorded for *Corymorpha*, among the lower animals (Child, '15*b*, pp. 142-146).

Unfortunately this culture of *Griffithsia* was made toward the end of the season, and my departure from Woods Hole prevented long continued observation. Through the kindness of Professor Osterhout, however, in turning over to me a culture of *Griffithsia* which had been in the laboratory some three months in very slowly running water, I was able to make some observations on more advanced stages of development of isolated cells. In this culture cell separation had occurred to a large extent, and many isolated cells showed considerable growth, although at the time of examination in many cases the new growths were dying or not in good condition. Figs. 7-10 show cases from this culture in which the susceptibility gradient was determined.

In Fig. 7 a new two-celled axis has arisen from the basal end of a cell, and the general susceptibility gradient, as indicated by the arrow, shows the usual relation to this axis, but the intracellular gradient in the large old cell is very slight.

Fig. 8 shows another case of a new axis consisting of three cells arising from the basal end of a large old cell and two new living rhizoids on the basal half and one dead rhizoid, indicated by

dotted lines, on the original apical region of the old cell. The arrows indicate the gradients. Death begins in the apical cell of the new axis and continues down the large cell to its middle,



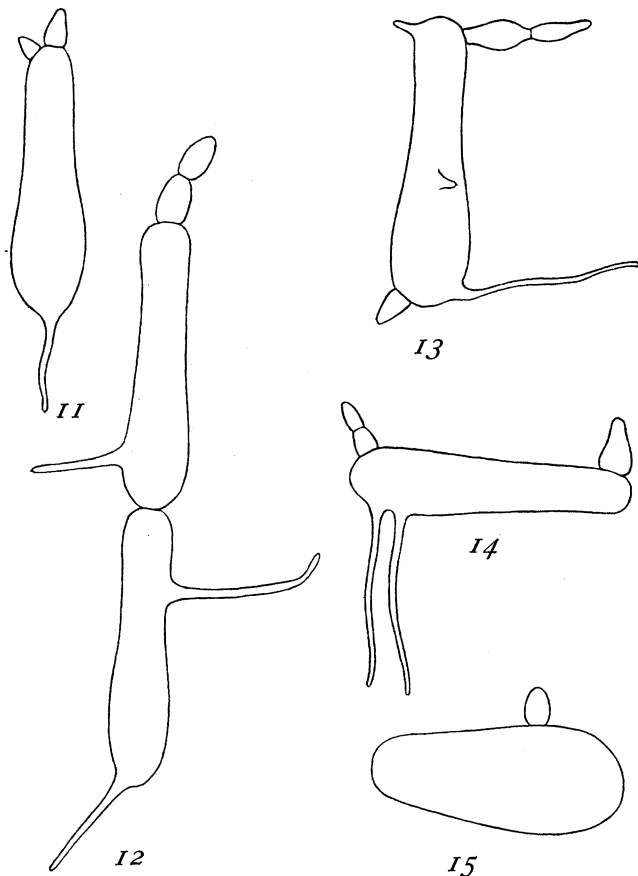
while an opposed gradient exists at the opposite end of the large cell. It is of course impossible to determine the physiological history of this cell, but appearances suggest that the cell was one of those in which the gradient had been reversed and a

rhizoid had arisen from the apical region before isolation (see p. 215). After isolation the conditions determined a double gradient, the old rhizoid died and new rhizoids arose in relation to the new axis. The original apical end of the cell, although it represents the high end of a gradient, is not active enough to give rise to a new apical cell.

Fig. 9 shows a cell with two new axes, one of four cells, the other of one cell, at its original basal end, one new two-celled axis at its apical end and a rhizoid arising from its middle region. The gradients are indicated by the arrows. It will be observed that a double gradient exists in the old cell, the middle region, where the rhizoid arises, being the region of lowest susceptibility. The two axes at the basal end and also the rhizoid were found to be more susceptible than the new axis at the apical end of the old cell, and the old cell is least susceptible of all, and its middle region the least susceptible region.

Fig. 10 is another case with new axes at both ends of the old cell. At the original apical end is one three-celled axis with two one-celled branches and one rhizoid arising from its most basal cell. At the original basal end of the old cell is another axis of two cells and the old cell also bears a rhizoid near its apical end. In this case observation of the progress of death was interrupted, and the susceptibility gradients were observed only in part. Those observed are indicated by arrows. This case showed all indications of being in poor physiological condition. The small apical cells were very heavily pigmented and instead of being rounded were flattened basally, suggesting a low turgor. The protoplasm of the large cell had also undergone structural changes similar to those which precede death in KCN and other agents. The susceptibility of all the new axes, except the one rhizoid axis arising from the old cell, was somewhat lower than that of the old cell itself. In the absence of knowledge of earlier stages in the life history of this case it is impossible to determine the meaning of the susceptibility relations between new and old parts. In several other cases the new axes were found to be less susceptible than the old cell, and in every such case the new axis, or at least its apical cell was very deeply pigmented and flattened basally, and the protoplasm of

the old cell had undergone more or less structural change. Apparently all such cases have ceased to grow and are, at least in large measure, quiescent, if not slowly dying. Their susceptibility is in general very low. Since this condition unquestionably results from the unfavorable environment, the new axes have been inhibited or injured to a much greater degree than the



old cell because of the originally higher susceptibility associated with their higher metabolic rate. If this is the case the question at once arises, why cell separation has not again occurred. As a matter of fact cell separation was occasionally seen in the new axes, though not very frequently. Its infrequency, even where

the gradient is reversed, is probably due to the greater area between the cells which is a very general characteristic of the new parts developing under these conditions. The cell turgor is apparently less than under normal conditions, and the young cells therefore depart more or less from the spherical or ovoid form characteristic of young cells produced under normal conditions, and in consequence of this change the surfaces of contact become larger and separation occurs less readily.

Figs. 11-14 show other cases of development of isolated cells from Osterhout's culture, in which the susceptibility gradients were not determined. In Fig. 11 two new apical cells arise from the original basal end, and a rhizoid from the original apical end of the cell. In Fig. 12 a new axis of two cells has developed from the basal end of a group of two cells, and rhizoids appear at various levels on the old cells. In Fig. 13 the two ends of the old cell apparently develop independently of each other, each giving rise to a new axis and a rhizoid at approximately right angles to the original axis, and a third rhizoid appears in the middle region. Fig. 14 shows a somewhat similar case with a two-celled axis and two rhizoids developed from the apical region of the old cell and a one-celled axis from the basal region. It will be noted that in all these cases the new apical cells arise at or near one or both ends of the old cells. Among all the cells examined, both from Osterhout's and from my own culture, only one case was found (Fig. 15) in which a new apical cell arose from the middle region of the old cell. This case was returned to the culture after examination in the hope that further development would occur, but could not be found later. Numerous other cases of development occurred in the isolated cells of these two cultures, but those described give a fair idea of the variety of axial relations observed.

DISCUSSION.

If the axial susceptibility gradient is in any degree an indication of the general metabolic relations along the axis, and if these metabolic relations are effective factors in determining the axial order or pattern, then the apical cell represents the region of highest metabolic rate, and in the vigorous axis under good

environmental conditions a gradient of decreasing metabolic activity extends basally. The rhizoid itself is a similar gradient, and the internal condition which favors rhizoid-development is apparently a low metabolic rate, though external conditions may determine whether it actually develops or not. It is, in fact, like the stolon of the hydroid, *Tubularia*, a secondary gradient, originating as a local region of increased metabolic activity in consequence of partial physiological isolation (Child, '15*b*, pp. 91, 92), in which external factors probably, at least often, play a part (Child, '15*b*, pp. 132-134). The rhizoid, in short, represents a secondary, more or less subordinate gradient, originating at what may be called a certain metabolic level in the axis or between certain metabolic limits and probably developing in relation to certain external factors. This conception agrees well with the fact that the susceptibility of the rhizoid, even at its tip, is usually lower than that of the apical region of the main axis, while even at its base, its susceptibility is usually higher than that of the level from which it arose.

Rhizoid-formation is not necessarily limited to the extreme basal end of the axis, for as metabolic rate decreases with advancing age of the cells, other levels may attain a condition which permits rhizoids to develop, and rhizoids may therefore appear first at the base and later rhizoid-formation may progress acropetally along the physiologically older portions of the axis, as it actually does in various species of algæ (Tobler, '06) and to some extent and rather irregularly in *Griffithsia*. As regards their relations to the primary axial gradient the roots of the higher plants are probably similar to the rhizoids (Child, '15*b*, pp. 156-163), though their function is of course different.

Assuming the correctness of this conception of the simplest plant axis, we should expect to find that in cells isolated from a preëxisting axis new apical cells would arise from the region of the cell possessing the highest metabolic rate and rhizoids from the region of lowest rate, or at least from lower metabolic levels than the apical cell. So far as determined, the susceptibility gradients in the forms developed from isolated cells agree in general with this expectation. In every case from my own culture, where only the earlier stages of new axial development

were present, the new apical cell arises from the region of highest susceptibility in the old cell. In the more advanced stages of development in the Osterhout culture most of the cases in which the susceptibility gradients were determined show the same relations, but some exceptions occur, apparently in cases which are in poor condition or gradually dying. Moreover, the fact that in these isolated cells new apical cells arise more frequently from the basal end or from both ends of the old cell than from the apical end alone is undoubtedly connected with the fact that cell separation is associated with obliteration or reversal of the original axial gradient in the cells. The greater frequency of development of new apical cells at or near one or both ends of the old cell than in the middle region is probably also an expression of gradient relations. Where the intracellular gradient was primarily basipetal and is completely reversed, the basal end of the cell becomes physiologically apical, and morphological development follows the new metabolic relations. Where the original gradient is largely obliterated, the two ends of the cell which were the regions of connection with other cells are doubtless more affected than other parts of the cell by the change in conditions accompanying the separation and undergo a certain amount of regression and rejuvenescence, and so attain a higher metabolic rate (Child, '15*a*, Chap. X.). It is also possible that in some cells from the more basal regions, where secondary basal elongation is occurring, and where two opposed intracellular gradients are often present (see p. 215) before separation, the obliteration of these gradients is not complete at the time of separation, and the higher rate at the two ends is still further increased by separation, so that both ends become physiologically apical. And finally, where the gradient is reversed in the normal plant, as sometimes in the more basal cells, it may be again reversed, obliterated or almost obliterated at the time of separation with correspondingly different results in later development.

While it is impossible to follow the changes in the gradient in a particular cell and to correlate them with the later development, the high degree of constancy in the relation between the intracellular gradients and the localization of the new axes leaves

little room for doubt concerning the fundamental character of the relation.

The cases where the new axes arise from the lateral surface rather than the end of the cell, as in Figs. 6, 13, 14, or from the middle region, as in Fig. 15, cannot be definitely accounted for, but are doubtless connected with special external conditions, perhaps the position of the cell with respect to light, oxygen supply or other external factors, or perhaps with local metabolic differences in the cell produced in some other way. I believe, however, that if we could analyze these cases, we should find that they, like the others, are an expression of the existing gradient relations, however these may have been determined (cf. Tobler, '06).

The localization of the rhizoids also agrees in general with expectation as far as the data go. In general they arise from a relatively low metabolic level on the cells, but this level may be in the middle region (Fig. 9) or at or near one end (Figs. 5, 6, 11), or rhizoids may appear progressively over a considerable length of the cell or cells (Figs. 8 and 12).

As regards the facts of morphological development, these experiments add nothing of fundamental importance to those of Tobler ('02, '04, '06), but the determination of the relations between the susceptibility gradients and cell separation and the localization of new axes serves to throw some light on the physiological conditions concerned in this development and on the problems of physiological polarity and physiological integration or individuation.

These experiments suggest further possibilities of more exact control of the localization of new axes on isolated cells, such as the determination of the influence of the direct subsection of single cells to the differential action of external factors on the localization of new axes.

SUMMARY.

1. The original axial susceptibility gradient of the alga *Griffithsia* is more or less completely obliterated or reversed after one or two days of confinement in a small volume of water, and the larger the volume of living plant protoplasm introduced, the more rapid the changes.

2. Where the conditions of confinement are extreme, obliteration or reversal of the gradient is followed in one or two days by death with a basipetal gradient, but under less extreme conditions the alteration of the gradient is followed by the separation of the axis into individual cells or occasionally small cell groups, the separation beginning apically and progressing basipetally.

3. Obliteration or reversal of the original axial gradient, followed by cell separation occurs, though more slowly, when the plants are kept in open standing water and to a greater or less degree, but still more slowly, in slowly running water in diffuse daylight.

4. A variable percentage of the isolated cells dies, the death rate being in general highest in apical cells and decreasing with the level of the cell in the axis, at least to the middle levels, below which there is often little difference. The cells which do not die may undergo a new development, giving rise to new apical cells and so to new axes.

5. Determinations of the susceptibility gradients in these reproductive developments from isolated cells show that the new apical cell arises from the region of highest susceptibility in the old cell. This region is more commonly basal than apical, because the gradient is usually reversed at the time of cell separation, but both ends of the cell may give rise to new apical cells where double opposed gradients exist. Rhizoids, on the other hand, arise from low levels in the axial gradient.

6. These experiments show that the original polarity of the cells of *Griffithsia* can be completely obliterated or reversed, and that the morphological development of new axes is an expression of gradient relations present in the cell at the time.

7. The facts support the conclusion that a gradient in metabolic rate, protoplasmic condition, or whatever we prefer to call it, of which the susceptibility gradient is within certain limits an indicator, constitutes physiological polarity in protoplasm, and that such a gradient is not an inherent property of protoplasm, but may be determined and altered by external factors.

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